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CAMSAPs Add to the Growing Microtubule Minus-End Story

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Free microtubule minus ends, found in many differentiated cells, contribute to polarized motility. Work from Jiang et al. (2014) in this issue of *Developmental Cell* shows how mammalian CAMSAP proteins stabilize minus ends, providing a key piece to the puzzle of how these minus ends are formed and stabilized.

Microtubules (MTs) in interphase cells are typically diagrammed as arrayed in a starburst pattern, with minus ends anchored at the centrosome and plus ends extending in all directions toward the plasma membrane. This common picture does reflect the MT pattern in many cell types, but a very different organization exists in a number of differentiated, polarized cells, in which most MTs are untethered to centrosomes and exist instead in the cytoplasm with both plus and minus ends free (see Figure 1) (Bartolini and Gundersen, 2006). Free MTs are typically arranged in parallel or antiparallel arrays, where they can serve as tracks for movement of cargoes from one end of the cell to the other (Bartolini and Gundersen, 2006; Zhu and Kaverina, 2013). While we now know much about MT organization in differentiated cells, major unanswered questions revolve around how free MTs are generated, stabilized, and organized. In this issue of *Developmental Cell*, Jiang et al. (2014) show how CAMSAP family proteins regulate MT minus-end stability in mammalian cells and demonstrate roles for CAMSAPs in polarized cell locomotion.

While MT plus-end dynamic turnover has been well characterized in cells, the fate of free MT minus ends has been much more of a puzzle. Early studies suggested that free MT minus ends never polymerize and either exist in a nongrowing (pause) state or depolymerize, possibly causing the entire MT to disappear (Rodionov et al., 1999). Proteins regulating MT minus-end stability have not been well characterized, but recent work has identified a family of proteins called CAMSAPs that are likely to be the long-anticipated regulators of MT minus-end stability. Patronin, a *Drosophila* protein, was the first CAMSAP identified. Patronin appears to cap MT minus ends and prevent tubulin addition to them. Patronin also stabilizes MT minus ends by protecting them from depolymerases such as kinesin 13, which can promote microtubule disassembly from either MT end (Goodwin and Vale, 2010). Mammalian CAMSAPs, including CAMSAP1, CAMSAP2, and CAMSAP3, are mammalian proteins related to patronin. Jiang et al. (2014) now provide a thorough examination of CAMSAP functions in vitro using purified proteins and cell-based assays to outline the function of each CAMSAP protein. These studies provide several surprises, including the slow polymerization of CAMSAP2-stabilized MT minus ends, which is unexpected given previous observations suggesting that minus ends do not polymerize.

CAMSAP2 is the most abundantly expressed CAMSAP in the cell lines examined by Jiang et al. (2014) and is the focus of much of their analyses. CAMSAP2 binds as a 1-μm-long stretch of proteins at the minus ends of free MTs. To study free minus ends, Jiang et al. (2014) generated them experimentally, either by laser microsurgery to sever existing MTs or by the spontaneous cytoplasmic nucleation that occurs shortly after washout of nocodazole, an MT-depolymerizing drug. CAMSAP2 does not nucleate new MTs and does not act as a cap at MT minus ends because some of the CAMSAP2-bound MTs grow, albeit at a slow rate. This slow polymerization rate may have been undetected in previous experiments. CAMSAP2 appears to bind MT minus ends shortly after tubulin addition, where it remains bound for a significant fraction of time. One function of CAMSAP2 is clear from its depletion: free minus ends always depolymerize. CAMSAP2 stretches bound to MTs can also act as stabilizers at the opposite end of the CAMSAP2 belt; depolymerizing plus ends switch back to growth when they encounter CAMSAP2 (Figure 1).

The slow turnover of CAMSAP2, combined with its addition to growing MT minus ends, led to the prediction that CAMSAP2 stretches should grow to longer lengths as MT minus ends elongate, but this was not observed. Surprisingly, Jiang et al. (2014) found that katanin, previously characterized as an MT-severing protein, maintains CAMSAP2 cluster length, somehow acting to remove MT-bound CAMSAP2 from the MT lattice (Figure 1). Without katanin, CAMSAP2 stretches grow to nearly triple their typical length. The mechanism underlying katanin regulation of CAMSAP2 binding to MTs is presently unknown.

The two other mammalian CAMSAPs, CAMSAP1 and CAMSAP3, also bind MT minus ends. CAMSAP1 does not stabilize MT minus ends but binds at the very tip of minus ends and appears to tip-track on these ends as they polymerize, making CAMSAP1 the first "-TIP," possibly functioning analogously to MT plus-end tracking, "+TIP," proteins (Akhmanova and Steinmetz, 2008). CAMSAP3, primarily studied in vitro by Jiang et al. (2014), binds to MT minus ends in much the same way as CAMSAP2, but likely with higher affinity. Although CAMSAP3 is the homolog of *Drosophila* patronin, it did not act as a minus-end cap and allowed slow minus-end polymerization.

Beyond simply describing the ability of CAMSAP2 and CAMSAP3 to stabilize MT minus ends, CAMSAPs are likely to

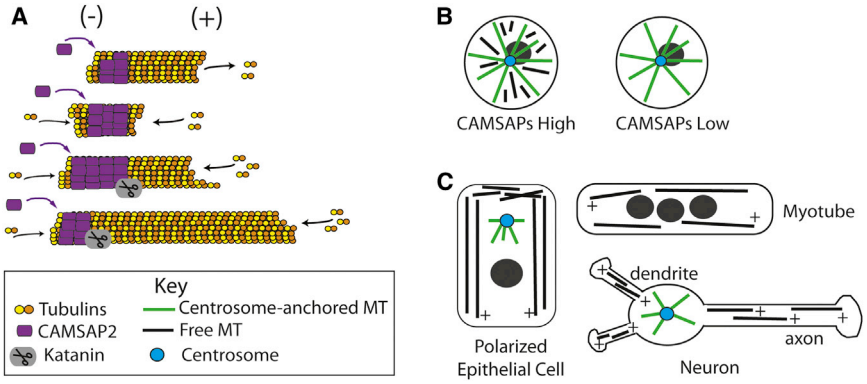

Figure 1. CAMSAPs Stabilize MT Minus Ends

(A) Schematic diagram of CAMSAP2 addition to MT minus ends coincident, or slightly after, tubulin addition. CAMSAP2 binds relatively strongly to the MT lattice, and its length along MTs is controlled by katanin. CAMSAP2 bound to MTs also acts as a stabilizer on the opposite end, allowing depolymerizing plus ends to resume growth. (B) CAMSAP2 and/or CAMSAP3 levels dictate the type of MT array formed. Higher concentrations of CAMSAPs favor free, unanchored MTs while lower concentrations favor centrosome-anchored MTs. (C) Examples of free and centrosomal-anchored MT organizations in differentiated, polarized cell types.

have critical and novel functions in regulating the organization of MT arrays and the cell functions that depend on these different MT arrangements. CAMSAP2 is necessary to maintain noncentrosomal MTs in cells. In the absence of CAMSAP2, MT organization is strictly radial, and all MTs are anchored at the centrosome. Thus, the abundance of CAMSAP2, and possibly CAMSAP3, could dictate the type of array assembled by a cell, in which a high concentration of CAMSAP2/3 favors free MTs and a lower concentration of CAMSAP2/3 favors a radial organization. It will be interesting to learn whether the morphological changes that occur as cells polarize and differentiate (see Figure 1C) require upregulation of CAMSAP2/3 expression. CAMSAP2 associates with MTs marked by detyrosination of α-tubulin, a posttranslational modification that marks long-lived MTs. Depletion of CAMSAP2 abolishes this population of posttranslationally modified MTs, likely by loss of MT minus-end stabilization (Jiang et al., 2014). However, two previous studies showed that CAMSAP3 depletion increased, rather than decreased, detyrosinated MTs (Nagae et al., 2013; Tanaka et al., 2012). Although CAMSAP2 and CAMSAP3 appear to act differently on tubulin detyrosination, these studies suggest that this tubulin posttranslational modification occurs on free MTs and possibly not on those MTs anchored to the centrosome.

The cellular consequences of free MT stabilization by CAMSAP2 are illustrated by its requirement in cell polarization and motility. In an artificial wound-healing assay, cells depleted of CAMSAP2 failed to polarize in response to the "wound," could not reorient the Golgi toward the wound edge, and showed poor migration into the cell-free area (Jiang et al., 2014). Curiously, MTs nucleated at the Golgi have also been implicated in cell polarization and migration in wound-healing assays (Zhu and Kaverina, 2013), raising the possibility that CAMSAPs function to stabilize those MTs initially nucleated at the Golgi, not at the centrosome. Now that CAMSAP functions have been described and the tools to localize them or regulate their expression level have been developed, any number of questions can be addressed in order to understand how free MTs contribute to cell polarity and the processes that depend on that polarity.

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